

Characterization of a New Enterococcal Gene, *satG*, Encoding a Putative Acetyltransferase Conferring Resistance to Streptogramin A Compounds

Streptogramin antibiotics are mixtures of two chemically unrelated A and B compounds that act synergistically in vivo against gram-positive pathogens, such as staphylococci, streptococci, and enterococci (8, 11). Resistance against B compounds is very widespread among enterococci and is mediated via the *ermB* gene cluster (e.g., on Tn917) that confers macrolide-lincosamide-streptogramin B resistance (7). The synergistic mixture of streptogramins A and B overcomes resistance to B compounds but is inactive in resistance to A compounds. The only known resistance mechanism against streptogramin A compounds in enterococci is mediated by the streptogramin acetyltransferase *SatA* (9). *Enterococcus faecium* isolates with *satA*-mediated resistance have been found in samples of human and animal origins, indicating a possible spread of resistance genes or resistant bacteria among different ecosystems (10).

We isolated a quinupristin-dalfopristin-resistant *E. faecium* UW1965 from a sewage treatment plant in Germany. The resistance determinant was transferred to a susceptible recipient, producing the transconjugant UW1965K1. UW1965K1 is resistant to quinupristin-dalfopristin (MIC ≥ 16 μ g/ml) and virginiamycin M (A compound; MIC, 16 μ g/ml), whereas the

MIC of each antibiotic for the recipient was 1 μ g/ml. PCR amplification for the *satA* gene was negative.

In staphylococci, resistance to streptogramin A compounds is mediated by two mechanisms: (i) acetylation of the streptogramin A via acetyltransferases (*Vat*, *VatB*, and *VatC* [1-3]) and (ii) efflux due to an ABC transporter (*Vga* and *VgaB* [4, 5]). PCR amplification for the *vat*, *vatB*, *vatC*, and *vga* genes failed to produce any product. The putative protein sequences of the known streptogramin acetyltransferases in staphylococci and enterococci contain three conserved motifs (2). Corresponding primers, *satI* and *satJ*, have been made, producing a 144- to 147-bp fragment for *vat*, *satA*, and *vatB* (2). PCR performed with these primers resulted in a ca. 150-bp fragment for UW1965K1. A digoxigenin-labelled probe of the amplified fragment was prepared, hybridizing with a 5.5-kbp fragment of *EcoRI*-digested plasmid DNA from the transconjugant. The corresponding plasmid fragment was cloned into pUC18 and sequenced.

The resulting DNA sequence (Fig. 1) did not show significant identity with other gene sequences from GenBank on the DNA level (6). One suitable open reading frame (ORF) was found, giving rise to a putative 214-amino-acid (214-aa) pro-

```

1  cggtagccgg  ggatcctcta  gactataatt  aaaattaaat  aactcaattc  ggaggttacta
      start      primer satG1
61  acctgactat  acctgacgca  aatgcaatct  atcctaactc  agccatcaaa  gaggtgtgtc
      M T I P D A N A I Y P N S A I K E V V F
121 ttatcaagaa  cgtgatcaaa  agtcccaata  ttgaaattgg  ggactacacc  tattatgatb
      I K N V I K S P N I E Y G D Y F Y Y D D
181 acccagtaaa  tcccaccgat  ttgagaacac  acgttaccca  tcactatgaa  ttcttaggcg
      P V N P T D F E K H V T H H Y E F L G D
241 acaaatat  catcggtaaa  tttgtttcta  tcgccagtgg  cattgaattt  atcatgaacg
      K L I I G K F C S I A S G I E F I M M G
301 gtgccaacca  cgtaattgaa  ggtatttcga  cttatccatt  taatatatta  ggtggcgatt
      A M M V M K G I S T Y P F N I L G G D W
361 ggcaacaata  cactcctgaa  ctgactgatt  tgccgttgaa  aggtgatact  gtatgggaa
      Q Q Y T P E L T D L P L K G D T V V G M
421 atgacgtgtg  gtttgggcaa  aatgtgaccg  tcctaccagg  cgtaaaaata  ggtgacggtg
      D V W V G Q N V T V L P G V K I G D G A
481 ccattatcgg  agcaaatagt  gttgtaacaa  aagacgtcgc  tccatatata  attgtcggcg
      I I G A N S V V T K D V A P Y T I V G G
541 gcaatccaat  tcaactcctc  ggaccaagat  ttgaaccgga  agttattcaa  gcattagaaa
      N P I Q L I G P R F E P E V I Q A L E N
601 atctggcatg  gtggaataaa  gatattgaat  ggataactgc  taatgttcct  aaactaatgc
      L A W W N K D I E W I T A N V P K L M Q
661 aaacaacacc  cacacttgaa  ttgataaaca  gtttaatgga  aaatcogaaa  caaaaaagcc
      T T P T L E L I N S L M E K
721 gtgcaagcaa  tccaaaaatg  attgtttaca  cggcctttac  tatttagtga  atccaattta
781 ttaataatag  atatgatata  ccagtaaaaa  atacactagc  cacctctggc  ggtactctac
841 tcgtatat  tatttacgac  cttctgatga  taaaggtcac  ttccctgtcc  ccagaaaaata
901 aagc
  
```

FIG. 1. A 904-bp sequence located on the 5.5-kbp cloned fragment in pUC18 (GenBank accession no. AF139725). The ORF begins at nucleotide 63 with an ATG start codon preceding a putative ribosomal binding site (RBS) (double-underlined) at positions 50 to 57. The predicted gene sequence encodes a protein of 214 aa which shows significant homology with other streptogramin acetyltransferases (aa motifs I, II, III; see also Fig. 2). The locations of the primers *satG1* and *satG2*, specific only for the *satG* sequence, are underlined (plus strand).

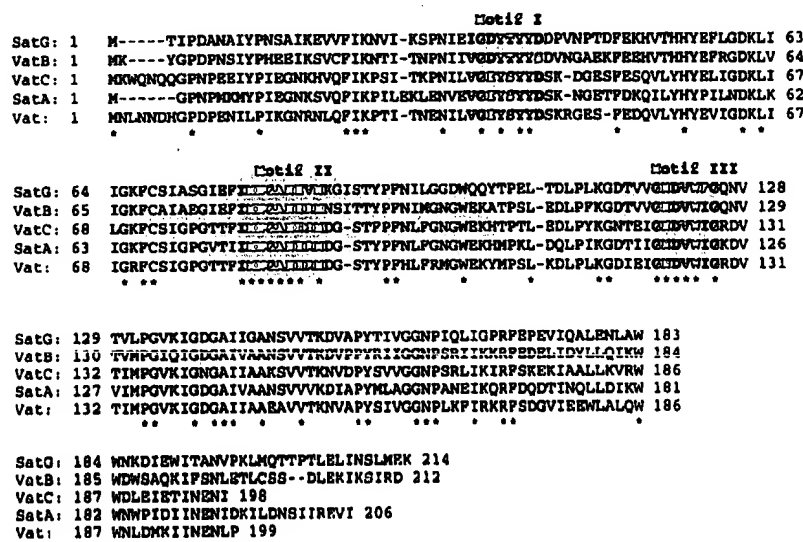


FIG. 2. Alignment of amino acid sequences of acetyltransferases from staphylococci and enterococci (1-3, 9) conferring resistance to streptogramin A antibiotics. Identical residues are indicated by asterisks. Highly conserved regions in different streptogramin A acetyltransferases—motifs I, II, and III—are boldfaced. Primers satI and satJ have been designed on the basis of the corresponding nucleotide sequences in motifs II and III (2).

tein. A comparison of amino acid similarities indicated rather significant homology between streptogramin acetyltransferases and the new putative acetyltransferase, designated SatG (Fig. 2). Based on the sequence for *satG*, two primers, satG1 and satG2, have been designed. Preliminary results of a search for streptogramin-resistant enterococci (*E. faecium*, *E. hirae*, and *E. durans*) revealed the existence of the *satG* gene in 9 of 23 isolates from sewage, 6 of 24 isolates from broiler samples, and all 17 isolates from poultry manure. Of 62 quinupristin-dalfopristin-resistant *E. faecium* (QDREF) isolates from hospitals in Germany, 9 were positive for *satG*. The high number of *satG* QDREF isolates from poultry meat and manure may be due to selection of these bacteria by use of virginiamycin as a feed additive, and spread of the resistance via the food chain to humans is very likely. This hypothesis is being investigated.

REFERENCES

- Alliget, J., V. Loncle, C. Stamenet, M. Dakepterre, and N. El Solh. 1993. Sequence of a staphylococcal gene, *vat*, encoding an acetyltransferase inactivating the A-type compounds of virginiamycin-like antibiotics. *Gene* 130: 91-98.
- Alliget, J., and N. El Solh. 1995. Diversity among the gram-positive acetyltransferases inactivating streptogramin A and structurally related compounds and characterization of a new staphylococcal determinant, *vatB*. *Antimicrob. Agents Chemother.* 39:2027-2029.
- Alliget, J., N. Llasove, and N. El Solh. 1998. Characterization of a staphylococcal plasmid related to pUB110 and carrying two novel genes, *vatC* and *vatD*, encoding resistance to streptogramins A and B and similar antibiotics. *Antimicrob. Agents Chemother.* 42:1794-1798.
- Alliget, J., V. Loncle, and N. El Solh. 1992. Sequence of a staphylococcal plasmid gene, *vga*, encoding a putative ATP-binding protein involved in resistance to virginiamycin A-like antibiotics. *Gene* 117:45-51.
- Alliget, J., and N. El Solh. 1997. Characterization of a new staphylococcal gene, *vgaB*, encoding a putative ABC transporter conferring resistance to streptogramin A and related compounds. *Gene* 202:133-138.
- Altchul, S., T. Madden, A. Schöler, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389-3402.
- Arthur, M., A. Brisson-Noël, and P. Courvalin. 1987. Origin and evolution of genes specifying resistance to macrolide, lincosamide, and streptogramin antibiotics: data and hypothesis. *J. Antimicrob. Chemother.* 20:783-802.
- Bouillo, H. J., M. B. Perri, C. A. Kouchman, and M. J. Zervos. 1996. Comparative *in-vitro* activity of quinupristin/dalfopristin against multidrug-resistant *Enterococcus faecium*. *Diagn. Microbiol. Infect. Dis.* 25:127-131.
- Rende-Fournier, R., R. Leclercq, M. Gallmand, J. Duval, and P. Courvalin. 1993. Identification of the *satA* gene encoding a streptogramin A acetyltransferase in *Enterococcus faecium* BM4145. *Antimicrob. Agents Chemother.* 37:2119-2125.
- Werner, G., I. Klare, and W. Witte. 1998. Association between quinupristin/dalfopristin resistance in glycopeptide-resistant *Enterococcus faecium* and the use of additives in animal feed. *Eur. J. Clin. Microbiol. Infect. Dis.* 17:401-402.
- Zervos, M. J. 1996. Vancomycin-resistant *Enterococcus faecium* infections in the ICU and quinupristin/dalfopristin. *New Horizons* 4:385-392.

G. Werner

W. Witte

Robert Koch Institute Wernigerode Branch
 Burgstrasse 37
 D-38855 Wernigerode
 Germany